

Sperm Development in the Imaginal Testes of *Aleiodes coxalis* (Hymenoptera: Braconidae: Rogadinae)

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Abstract—Spermatogenesis and spermiogenesis in the imaginal testes of the long-lived braconid wasp, *Aleiodes coxalis* (Spinola) has been investigated. The cyclostome group of braconid subfamilies, to which *Aleiodes* belongs, are considered to have unspecialised sperm, but in *Aleiodes* several aspects of spermatogenesis and morphology differ from that reported in other Hymenoptera, suggesting that reinterpretation of previously published studies may be necessary. In particular, the centriolar adjunct is found to lie between the nucleus and one of the pair of mitochondrial derivatives, resulting in the mitochondrial derivatives being offset longitudinally, giving the impression that the mitochondrial derivatives are of different lengths. A projection extends from the centriolar adjunct to the one mitochondrial derivative which abuts the nucleus. The shape change undergone by the nucleus during spermatogenesis is associated with an uneven distribution of peri-nuclear microtubules (microtubular manchette). These are not found over two extra-nuclear electron-dense regions, ('lateral plates') which appear to add increased rigidity to the nucleus at these points.

Very little is known about the sperm of Hymenoptera despite the fact that this is one of the largest and most diverse of insect orders (Jamieson 1987), and descriptions of spermatogenesis are limited to a handful of taxa, mostly bees and ants (Hoage & Kessel 1968, Hogge & King 1975, Cruz-Landim & Beig 1980). A recent survey of mature sperm morphology and ultrastructure across the order has revealed considerable variation between higher taxa that may be important for understanding phylogenetic relationships within the order (Quicke et al. 1992), some of the most extreme modifications occurring in the parasitic wasp family, Braconidae. In order to interpret the structures and modifications found in some braconid subfamilies it is first necessary to describe the 'normal' situation in that family.

The Braconidae is a large family of parasitic wasps comprising some 40 subfam-

ilies that can be broadly divided into two groups, the cyclostomes and the non-cyclostomes, which differ in morphology of the mouth and in their biology (Shaw & Huddleston 1991). In an initial survey (Quicke et al. 1992), it was found that in members of the cyclostome group of braconid subfamilies (e.g. Braconinae, Doryctinae and Rogadinae, the latter including *Aleiodes*) the sperm have a normal appearance, being between 80 and 170 μm long with the head (nucleus + acrosome) comprising 10 to 20% of the sperm's total length. However, nearly all non-cyclostomes have apomorphic sperm with total length between 10 and 20 μm , of which the head constitutes nearly 50%. Further, whilst the axonemes of the cyclostome braconid sperm have a normal 9+9+2 arrangement of microtubules, as found in most insects, those of the short apomorphic non-cyclostome sperm frequently have only one or no central microtubules. These features appear to be highly phy-

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logenetically informative and, in order better to understand their ontogenies, we have investigated spermatogenesis in a range of braconids. Here we report on that leading to the apparently unmodified sperm of the rogadine, *Aleiodes coxalis* (Spinola), which will form the basis for future comparisons. Despite the normal appearance and ultrastructure of the mature sperm in *Aleiodes*, spermatogenesis in this taxon exhibited several structures that do not appear to have been reported in other insects.

It has long been appreciated that the timing of spermiogenesis and spermatogenesis in insect testes is a reflection of the adult life span which may in turn be associated with the occurrence of sib-mating (Phillips 1970). Thus imaginal testes of species with short lived adults, such as mayflies (Needham et al. 1935) and caddis flies (Ross 1944), or those in which males almost invariably mate with their nearby sisters upon emergence such as many parasitic wasps, typically contain spermatids and spermatozoa, but no gonial and meiotic divisions, which would be completed in the pupal or nymphal stages. In contrast, meiotic and even gonial divisions often continue well into the imaginal stages in testes of long lived species (e.g. many beetles and dragonflies) which usually search for and mate with several unrelated females. Most rogadine braconid wasps, such as the *Aleiodes* species investigated here, are solitary parasitoids of Lepidoptera larvae and belong to the second category. Males fed on honey water will often live a month and sometimes considerably longer, and will mate readily throughout much of their adult life (M. R. Shaw pers. comm.). Indeed, some species are reluctant to mate immediately after eclosion and only start to mate once they have fed.

There are no papers on the ultrastructure of spermatogenesis in any member of the Ichneumonoidea, and the only parasitic Hymenopteran studied to date is the

pteromalid chalcidoid *Nasonia vitripennis* (Walker) (Hogge & King 1975).

MATERIALS AND METHODS

Adult *Aleiodes coxalis* (Spinola) were collected at Silwood Park (Imperial College) in 1995. Testes were dissected under 2% glutaraldehyde in phosphate buffered saline (pH 7.2), and fixed for two hours. Tissue was transferred to 2% osmium tetroxide in cacodylate buffer (pH 7.2) for 2 hr. After another buffer wash, tissue pieces were dehydrated to 50% ethanol and then further fixed with saturated uranyl acetate in 50% ethanol prior to complete dehydration, embedding in Epon resin and polymerisation overnight. Large silver sections were picked-up onto high resolution grids, stained with uranyl acetate and lead citrate and examined using a Phillips EM400 electron microscope.

RESULTS

Adult testes of *Aleiodes coxalis* contain follicles (seminal tubules) with multiple cysts present at various levels of maturity. Although neighbouring cysts can exhibit different stages of development, generally the more posterior the position along the testes the greater the proportion of later stages. Although most cysts contain spermatids, it is also possible to find in the adult indications of earlier stages of development. Primary spermatocytes still appear to be present some without any sign of a normal spindle and with a concentric arrangement of the endoplasmic reticulum around the chromosomes (Fig. 1 long arrow). Centriolar pairs are present in these cells (arrowheads), as are large numbers of small mitochondria. This appearance is considered indicative of an abortive first meiotic division; the result of incomplete centriolar polarisation. Centrioles are then extruded after meiotic multiplication by cytoplasmic blebbing (Fig. 2), where centrioles, attached to microtubules (Fig. 2a), are found incorporated into rounded portions of the plasma mem-

brane. This blebbing is also seen in secondary spermatocytes (which also exhibit loss of plasma membrane in larger whorls), although not all blebs exhibit centrioles (Fig. 2b *arrow*). Large numbers of polyribosomes are also present at this stage.

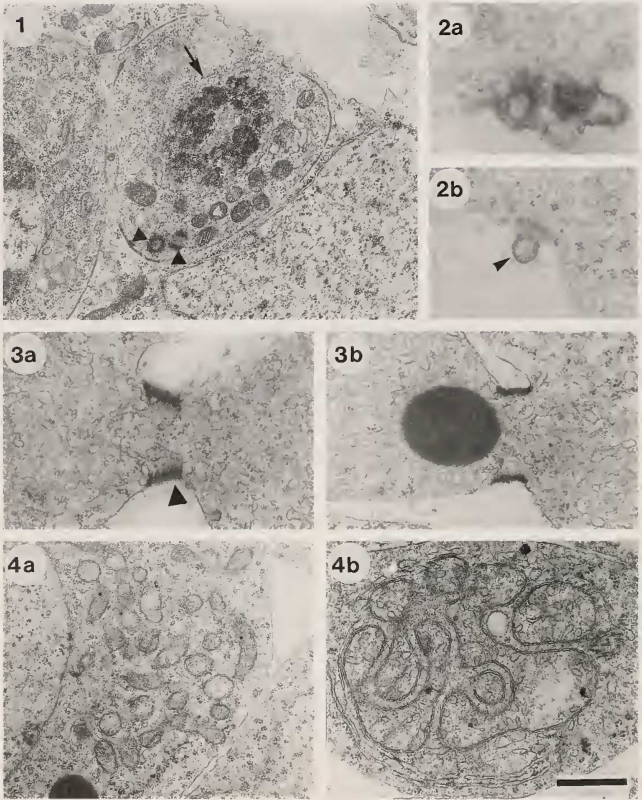
In the secondary spermatocytes it is difficult to find evidence of synaptonemal-like structures. At this stage the cells are considered to form a syncytium because of incomplete cytogenesis during the earlier mitotic division. The intercellular bridges (Fig. 3) linking the spermatocytes are difficult to identify as the cells can be very closely apposed. It is only during later spermiogenesis, with cell elongation, that the connections become apparent (Fig. 3a) with evidence of organelle continuity and possibly even intercellular movement of vesicles and granules (Fig. 3b). This has been suggested as being a method of transferring organelles from large to small spermatids where there is unequal division after the second meiotic division. No noticeable size difference was seen in *Aleiodes*.

Nebenkern formation occurs concomitantly with the above processes (Fig. 4). Mitochondria begin to accumulate in one area of the cell (Fig. 4a) where they fuse to form the beginnings of the nebenkern (Fig. 4b), a highly convoluted membranous organelle that is the precursor of the two mitochondrial derivatives. The development of the nebenkern into two labyrinthian networks has been linked with the appearance of microtubules in the cytoplasm. However, in *Aleiodes* microtubules appear to be a consistent component of the cytoplasm from earlier stages.

Flagellum growth (Fig. 5) is evident prior to nuclear condensation, commencing with the appearance of a basal body (Fig. 5a *arrowhead*) in a small depression of the nucleus (Fig. 5a *arrow*). The nuclear envelope thickens at this area (Fig. 5b) and the doublet microtubules of the flagellum grow from the triplet microtubules of the

basal body (Fig. 5b *arrow*). The flagella initially do not have accessory tubules (Fig. 5c). These appear to develop from sub-fibre b of each doublet as previously reported. The flagella/mitochondria axis is developed before this takes place (Fig. 5d *arrow*). Although spermiogenesis is advanced at this stage, separation following the last meiotic division in many cases is not complete and the nucleus still appears spherical. Nuclear condensation begins with the appearance of polarity in the nuclear membrane in the region closely apposed to the spermatid cell membrane (Figs 5d, 6 *arrowheads*) where the spermatid is attached to the wall of the cyst (a single epithelial layer with supportive and secretory functions). Microtubules appear around the nucleus but these are not evenly located; an arrangement which probably assists the complicated shape change that the nucleus undergoes.

The acrosome is detectable in early spermatid stages as an accumulation of a large number of small particles termed the proacrosomal granule (Fig. 7). During spermiogenesis the proacrosomal granule becomes located between the plasma membrane and the nucleus at the region of nuclear polarity (Fig. 8a). The acrosome is formed by a flattening of this structure and an indenting on the side apposed to the nucleus (Fig. 8b). A small protrusion is found anteriorly to the acrosome in the early spermatid (Fig. 8b *arrow*). This may represent an apical expansion of extra-acrosomal material, similar to that found in *Locusta migratoria* L. (Szöllösi 1974). However, the structure in *Aleiodes* appears to contain electron dense material. Whether this could be a separate structure, or represents a later stage of fusion of Golgi derived products, as found in the proacrosomal granule, is not clear. The situation is further complicated by the impression that the is distinct coated membrane which surrounds the acrosome and extends posteriorly over the anterior portion of the nucleus during development, at



Figs. 1-4. 1, Primary spermatocyte from the testes of an adult male *Aleiodes*, with anomalous metaphase plate formation, characterised by the absence of a normal spindle and a concentric arrangement of the endoplasmic reticulum around the chromosomes (arrow). Large numbers of small mitochondria and a centriolar pair (arrowheads), are also present. Scale bar = $0.5 \mu\text{m}$. 2, Membrane blebbing during spermiogenesis: (a) loss of centrioles (attached to microtubules) through membrane blebbing; (b) membrane blebbing (arrow) without loss of centrioles. Scale bar a = $0.6 \mu\text{m}$; b = $0.85 \mu\text{m}$. 3, Syncytium formed by incomplete cytokinesis during mitotic division: (a) Intercellular bridges (arrowhead) link spermatocytes; (b) linkage provides a method for organelle movement between spermatocytes. Scale bar = $0.75 \mu\text{m}$. 4, Nebenkern formation in the spermatocyte.

least initially does not appear to be continuous with this granular tip. As the acrosome elongates an acrosomal rod (or perforatorium) develops, and extends from a depression in the nucleus and into the subacrosomal space formed during folding (Fig. 8c, *arrow*). The acrosomal rod becomes hollow as it develops (Fig. 8d), but does not occupy all of the large sub-acrosomal space (Fig. 8e). At this stage the complete structure is referred to as the acrosomal complex.

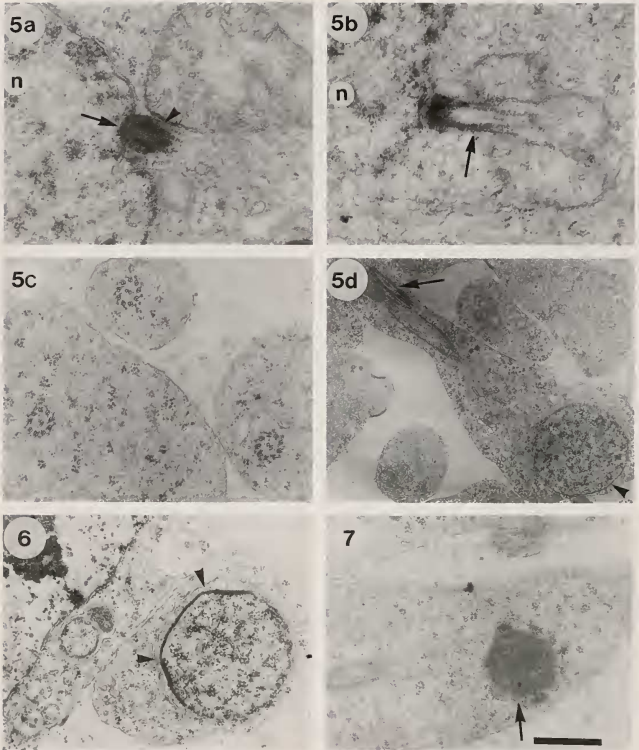
With the formation of the acrosomal complex and cell elongation, a distinct centriolar-adjunct appears in the spermatocyte (Fig. 9). It develops at the posterior nuclear pole adjacent to the forming basal body (Fig. 9a). Unlike the situation in some other insect species (Gatenby & Tahmisian 1959, Breland et al. 1966) it does not appear to be forming around the centriole that is perpendicular to the axis of the flagellum. Instead, the basal body is located between the centriolar adjunct and the 'perpendicular' centriole; the ultimate fate of this second centriole is not clear. In transverse section, the axoneme is associated with one mitochondrial derivative and the sectioned centriolar adjunct (which in the past has been interpreted as a darkened mitochondrial derivative). In longitudinal section the centriolar adjunct can be seen to lie between the posterior pole of the nucleus and one of the mitochondrial derivatives (Fig. 9c), and is slightly more than $2\mu\text{m}$ long. The mitochondrial derivatives are thus offset longitudinally, with the other member of the pair abutting the nucleus. This may result in the appearance of the adult mitochondrial derivatives, where one member of the pair often seems to extend further posteriorly, possibly giving the erroneous im-

pression that in fact the mitochondrial derivatives are of different lengths. This is a different arrangement from that previously reported for other related species. Furthermore, anteriorly, it can be seen that for part of the length of the centriolar adjunct, where the centriolar adjunct and one of mitochondrial derivatives lie side by side, there is an extension of the centriolar adjunct which surrounds the mitochondrial derivative on the side facing the centriolar adjunct (Fig. 9d). Interestingly in a rare teratological sperm-tail with two axonemes the centriolar adjunct was found to extend to enclose two mitochondrial derivatives. This contact is most evident in the region of the basal body and it is not clear if it actually extends into the flagellum proper as defined by the possession of both a ring of doublet microtubules and a central pair of microtubules.

The change in shape of the nucleus (Fig. 10) that occurs with the appearance of peri-nuclear microtubules is also marked by the formation of two extra-nuclear electron-dense regions (Fig. 10a, see also Fig. 6). Peri-nuclear microtubules are unevenly distributed and are not found over the extra-nuclear electron-dense regions, which we have called lateral plates, nor do they occur in the area of the nucleus that will be convex during the elongation process. The lateral plates appear to be the locus for shape changes that occur during the elongation of the nucleus. Condensation of the chromatin into coiled fibrillar threads then follows (Fig. 10b). In transverse section, as the nucleus elongates, the threads are found associated with that portion of the nuclear membrane that is adjacent to the region lacking microtubules (Fig. 10c). The side of the nuclear membrane surrounded by the full complement

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cyte: (a) Large numbers of small mitochondria accumulate in one area of the spermatocyte; (b) mitochondrial fusion occurs to form the labyrinthine network of the nebenkern, which will itself then give the two mitochondrial derivatives of the mature sperm. Scale bar a = $1.4\mu\text{m}$; b = $1.15\mu\text{m}$.



Figs. 5-7. 5, Flagellum formation during spermiogenesis: (a) the basal body (*arrowhead*) forms from the lateral centriole by insertion into a depression of the nucleus (*arrow*); (b) doublet microtubules (*arrow*) grow from the triplet microtubules of the basal body; (c) accessory tubules are absent at this stage, although a central pair of microtubules form in the developing axoneme post-basal body; (d) the flagella/mitochondria axis is developed (*arrow*) before the change in shape of the nucleus is found, although polarity in the nuclear membrane can be found. n = nucleus. Scale bar a, b = 0.7 μm ; c = 0.8 μm ; d = 3 μm . 6, Polarity of the nucleus develops prior to shape change with increased electron density of the nuclear membrane including the appearance of two extra-nuclear densities (lateral plates). Microtubules (*arrowheads*) appear around the nucleus. Scale bar = 1 μm . 7, Proacrosomal granule formed from small Golgi derived vesicles. Scale bar = 1 μm .

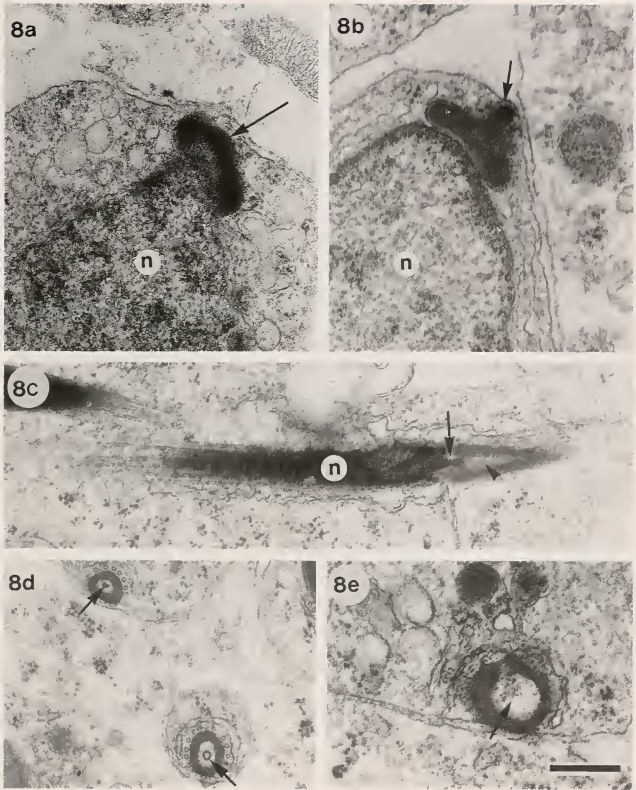


Fig. 8. Formation of the acrosomal complex during spermiogenesis: (a) the acrosome becomes located between the plasma membrane and the nucleus (arrow); (b) the acrosome indents to enfold the nucleus, and a small electron density is found anteriorly; (c) an acrosomal rod ('perforatorium' arrow) occupies the subacrosomal space formed during folding; (d) the perforatorium (arrow) is seen in transverse section to be hollow; (e) the subacrosomal space itself can be seen to be large and is not filled by the perforatorium for its entire length. n, nucleus. Scale bar a, c, d = 0.5 μm ; b, e = 0.65 μm .

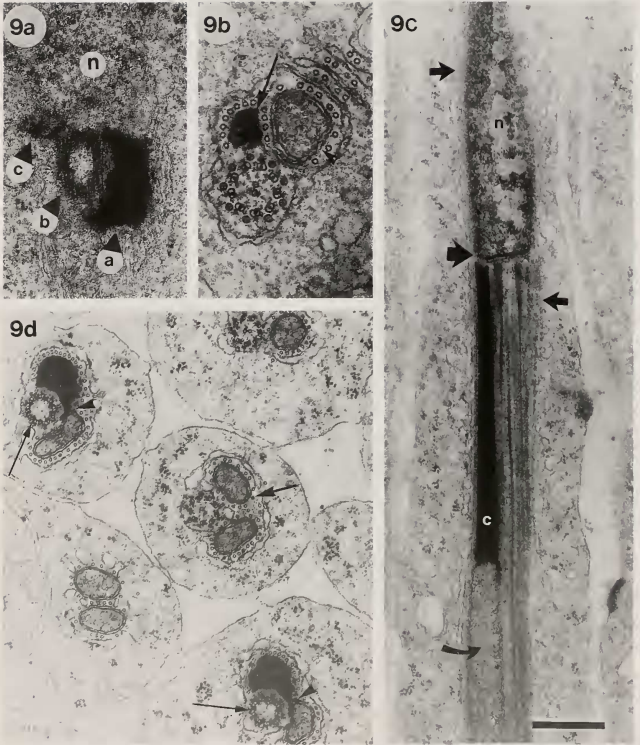


Fig. 9. Centriolar adjunct formation during spermiogenesis: (a) arrangement of centriolar adjunct (arrow, 'a'), basal body (arrow, 'b') and perpendicular centriole (arrow, 'c'); (b) in transverse section the centriolar adjunct (arrow) and a single mitochondrial derivative (arrowhead) lie in parallel with the axoneme; (c) in longitudinal section the centriolar adjunct (c) abuts the nucleus anteriorly at the nuclear plate (large arrow) and a mitochondrial derivative (curved arrow) posteriorly. Microtubules (arrows) are evident lining the nucleus (n) and the developing axoneme; (d) at the level of the basal body (arrows) the centriolar adjunct (arrowheads) extends to contact and enclose the single mitochondrial derivative. Posteriorly to the centriolar adjunct two mitochondrial derivatives are found (arrow). Note also the absence of the central pair of microtubules in the basal bodies. n, nucleus. Scale bar a = 0.5 μm ; b = 0.3 μm ; c = 0.6 μm ; d = 0.9 μm .

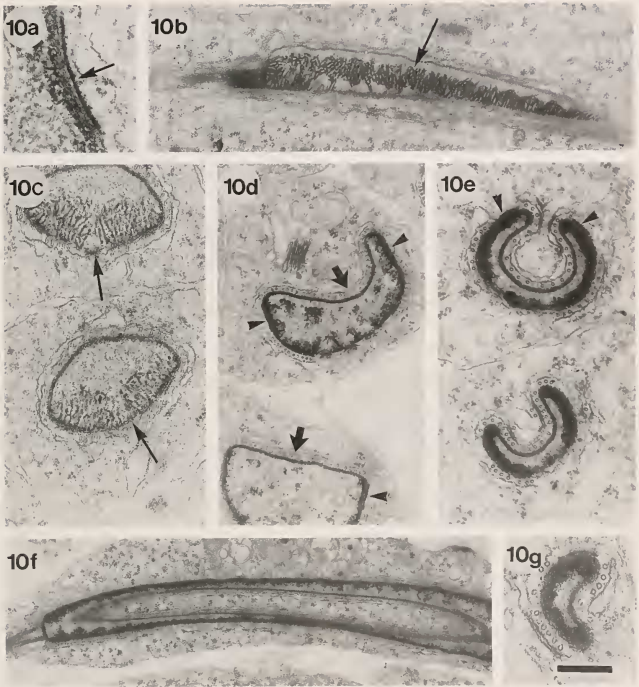


Fig. 10. Change in shape of nucleus during spermiogenesis: (a) extra-nuclear electron-dense region ('lateral plate', arrow) are produced upon nuclear shape-change; (b) condensation of the chromatin into coiled fibrillar threads (arrow); (c) the threads divide and become associated with that portion of the nuclear membrane on either side of the region lacking microtubules (arrows); (d) the side of the nucleus with the full complement of microtubules (large arrows) flattens, and then infolds with the lateral plates (arrowheads) providing a locus for shape change; (e) the chromatin becomes a layer on the outer surface of a horse shoe (arrowheads) that infolds to surround elements of the endoplasmic reticulum; (f) as the spermatocyte elongates the groove begins to disappear from the anterior and posterior ends; (g) the dense nuclear material then begins to redistribute more evenly around the nuclear membrane as the width decreases. Scale bar a = 40 μm ; b = 1 μm ; c, d, e = 0.6 μm ; f = 1.2 μm ; g = 1.7 μm .

of microtubules then flattens (Fig. 10d *arrow*), with the chromatin becoming a layer on the outer surface of the developing horse-shoe shape; as viewed in section (Fig. 10d *arrowhead*). The lateral plates become the linear portions of the outer surface of the arms of the 'horse-shoe' (Fig. 10e *arrowheads*); at the most extreme, the lateral arms (in transverse section) almost come into contact with one another. As the nucleus infolds, so elements of the endoplasmic reticulum, which surround the microtubules, become largely enclosed by the nucleus (Fig. 10e). It is not clear how this highly folded nucleus reaches the cylindrical shape of the adult sperm. As the sperm elongates it appears that the inverted side is flattened with the result that the groove begins to disappear from the anterior and posterior ends (Fig. 10f). The dense nuclear material then begins to redistribute more evenly around the nuclear membrane as the width decreases (Fig. 10g); however, intermediate stages are difficult to identify.

The axoneme itself develops into the 9+9+2 arrangement (Fig. 11a); 9 outer single accessory tubules, 9 doublets and 2 central single microtubules. Intratubular material is abundant (with radial spokes—Afzelius rays) and indications of the inner and outer dynein arms. Two deltoid bodies, (also referred to as triangular rods; Lensky et al. 1979) develop, but their exact derivation is not clear, although a membrane origin is apparent and a close association with the mitochondrial derivatives is probable. This is further supported by the observation that only one deltoid body is found at the level of the centriolar where there is only a single mitochondrial derivative. At the level of the two deltoid bodies is a single central rod, as previously reported in ant spermatozoa (Wheeler et al. 1990). This arrangement is maintained in mature sperm (Fig. 11b). By comparison with that of the spermatocyte, the nucleus of the mature sperm is homogeneous (Fig. 11c). The acrosome develops a

distinctive glycocalyx (Fig. 11c *arrowheads*) which extends over the anterior portion of the nucleus. The two mitochondrial derivatives show the offset arrangement that may be the result of the centriolar adjunct overlaying one of them anteriorly (Fig. 11c *arrows*). In addition an end piece with no mitochondrial derivatives is found (Fig. 11d *arrow*).

DISCUSSION

In the testes of imaginal male *Aleiodes* we have found structures indicative of early stages of sperm development. In particular, evidence for an abortive first meiotic division of the primary spermatocyte, a major feature of hymenopterous insects in which males are haploid, has been found. This appears to involve anomalous metaphase plate formation, probably caused by the absence of a normal spindle. This has been previously described in the drone of the bee *Scaptotrigona postica* by Cruz-Landim & Beig (1980), where it is characterised by the presence of a concentric arrangement of the endoplasmic reticulum around the chromosomes and the absence of a normal spindle. In *Aleiodes*, as in *S. postica*, this is connected to an anomalous movement of spermatocyte centrioles, which, instead of assuming polar locations, migrate to the cell surface and are lost by cytoplasmic blebbing (Hoage & Kessel 1968). The presence of such early stages of development in the adult supports our supposition that gonial development in this species could be related to the life style of the adult. The relatively long adult life time of the male would seem to obviate the need for synchronous maturation of all the individual sperm cysts.

The manchette of microtubules that assembles around the developing spermatid nucleus appear to be important for nuclear compression and elongation (Baccetti 1972). The nuclear shape change found during development in *Aleiodes*, and the asymmetric distribution of microtubules,

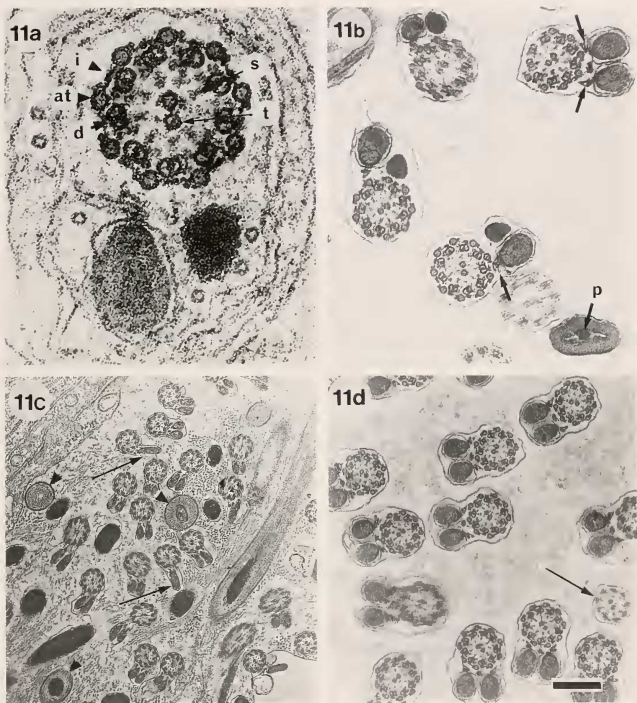


Fig. 11. Axoneme structure and the mature sperm: (a) The arrangement of 9 outer, singlet accessory microtubules (at), 9 doublet (d) and two single central microtubules (t) is evident in the later stages of spermiogenesis, with intratubular material (i) and prominent radial spikes (s); (b) two deltoid bodies (arrows) develop in association with the mitochondrial derivative except at the level of the centriolar adjunct where only one is found; (c) the mature sperm illustrates the uneven length of the mitochondrial derivatives (arrow) and the glycocalyx that develops around the mature acrosome (arrowheads); (d) the tail piece at the posterior of the sperm (arrow) contains only the 9+2 arrangement of doublet and single microtubules. Scale bar a = 100 μ m; b = 0.25 μ m; c = 0.75 μ m; d = 0.4 μ m; p = perforatorium.

have not previously been described for parasitic wasps. A gutter-shaped developmental stage of the nucleus occurs in some bugs (Lee & Lee 1992) but nuclear

elongation is not accompanied by concavity in many other insects (e.g. Szöllösi 1975, Friedländer 1993, Wolf & Joshi 1995). In some caddis flies, in which the

spermatid nucleus transiently assumes a sickle shape, the microtubular manchette surrounding the nucleus is interrupted (Wolf & Klein 1995), as it is in the dragonfly, *Aeshna grandis* L., in which the elongating nucleus becomes locally compressed (Kessel 1966). The structures we have termed lateral plates, which occur between groups of microtubules of the microtubular manchette, do not appear to have been described previously in any insect.

The present study of spermiogenesis in *Aleiodes* has implications for published studies of the centriolar adjunct. The presence of a centriolar adjunct overlying only one of the mitochondrial derivatives has not to our knowledge previously been reported. In particular the connection from the centriolar adjunct to the mitochondrial derivative that abuts the nucleus is novel. This arrangement may be responsible for observations in related species suggesting that anteriorly there may be a particularly electron dense mitochondrial derivative (Quicke et al. 1992) or a region of overlap between the axoneme, nucleus and mitochondrial derivative (Chauvin et al. 1987)—i.e. some studies may have misinterpreted the centriolar adjunct as a particularly electron dense mitochondrial derivative. Further, the longitudinal offsetting of the mitochondrial derivatives caused by the interposition of the centriolar adjunct between one of them and the nucleus could be responsible for the appearance of some sections through the posterior region of the sperm, just before the tail piece, in which only one mitochondrial derivative is found. The presence of a single mitochondrial derivative in sections through this region in other species prompted previous authors to conclude that mitochondrial derivatives are of different lengths, which has even been considered to be a characteristic, not just of the Hymenoptera, but of all holometabolous insect orders (Wheeler et al. 1990). However this result could be explained

simply by the arrangement of the centriolar adjunct. In the light of this finding in *Aleiodes*, it would be interesting to re-examine these previously reported groups for the presence of a similarly located centriolar adjunct. A large centriolar adjunct has been reported in ant spermatozoa (Wheeler et al. 1990), and was thought to distinguish this group from other Hymenoptera, such as bees, which appear to lack a homologous structure (Lensky et al. 1979, Cruz-Landim & Beig 1980). The arrangement of the centriolar adjunct in the ant spermatozoa, at least in longitudinal section, is similar to that of *Aleiodes*. However, in these the centriolar adjunct can be seen to overlie both mitochondrial derivatives.

The connection to the mitochondrial derivative is particularly interesting as it occurs at the level of the basal body where the two central microtubules are absent. The origin and exact function of the central pair is not known. A structural role for the central pair could imply a similar role for the centriolar adjunct and the latter could therefore be compensating for the absence of the central pair at the region of the basal body. However, some studies have provided evidence that the centriolar adjunct consists partly of RNA (Baccetti et al. 1969) and how this would be related to a structural role is uncertain.

The exact relationship between centrioles, the basal body, the centriolar adjunct and the developing axoneme in *Aleiodes* is not clear. In another parasitic wasp, the pteromalid *Nasonia vitripennis* (Walker), the distal centriole is reported to lengthen to form the basal body of the flagellum, and then, with the proximal centriole, form the ring centriole (Hogge & King 1975). This then associates with a spherical reticulate dense body, which forms adjacent to the nuclear membrane opposite a nuclear pore at the secondary spermatocyte stage. As maturation proceeds the proximal centriole is reported to become surrounded by granular material and

break down, with loss of material from the spherical, reticulate dense-body. The granular material, with presumably the remains of the proximal centriole is then thought to contribute to the centriolar adjunct. However, in *Aleiodes* such a straightforward relationship is not clear; in fact it appears as if the centriolar adjunct may develop prior to loss of the proximal centriole. Thus this may not contribute to the formation of the centriolar adjunct at all. In discussions of the centriolar adjunct in ant spermatozoa, Wheeler et al. (1990) suggest caution when applying this term, as the developmental homology to such structures in other species, such as mammals, has not been proven.

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